

**Studies on the Phenolic Constituents
of Babul Part I Affinity Studies**

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STUDIES ON THE PHENOLIC CONSTITUENTS OF BABUL* PART I AFFINITY STUDIES

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Among the phenolic constituents present in babul, (+)-leucocyanidin gallate and the two polymeric tan fractions T₁ and T₂ which form almost the entire tanning component were found to be responsible for the tanning potency of babul liquor, with fairly good affinity towards hide powder.

Babul bark (*Acacia arabica*) is one of the principal vegetable tan materials of India and considerable work on the different aspects of this material has already been carried out.¹⁻⁵ Bhanu *et al.*⁵ isolated a number of the phenolic constituents present in babul. Except for preparing some derivatives from the individual constituents isolated from babul in order to ascribe a definite structure, no work appears to have been carried out so far on the tanning potency of the individual phenolic constituents or on their affinity towards hide powder. The present paper will deal only with the work carried out on the tanning potency of some of the individual constituents isolated from babul as well as their affinity towards hide powder.

Experimental

Preparation of group fractions and individual constituents

One kg. of crushed babul bark was extracted with 5000 ml. acetone for 48 hours.

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After decanting the supernatant, the bark residue was again treated with 5000 ml. acetone and kept in that condition overnight and then filtered. This procedure was repeated once again. The acetone extracts were combined, filtered and dried *in vacuo* to dryness. The dried acetone extract was washed six times with petroleum ether (40-60°) for removing the fatty and other resinous materials and the extract was then, dried in the air to free it of petroleum ether. It was then repeatedly extracted with ether till all the ether solubles were removed and then with ethyl acetate till ethyl acetate solubles were removed completely. The ether and ethyl acetate solubles were distilled under vacuum, collected separately and dried.

The constituents of babul bark such as (+)-catechin, (-)-*epi*-catechin, (+)-dicatechin, quercetin, gallic acid, (+)-leucocyanidin gallate and two polymeric tan fractions T₁ (butanol solubles) and T₂ (butanol insolubles) were isolated as described by Bhanu *et al.*⁵ Except for the two polymeric tan fractions, all the

other constituents isolated were further purified by passing through polyamide column prior to start the tanning experiment. Since one of the constituents, quercetin was found to be insoluble in water, tanning studies with all these constituents and group fractions were carried out in acetone medium. 0.6% solution of all the constituents was made with acetone and sausage casings (intestine of cow),⁶ which were used as substrate were tanned in the solutions for 3 days. The T_s of the sausage casing was then determined after putting the casing in water for 3 hours.

Affinity of the constituents towards hide powder

One g. lots of hide powder were weighed accurately into 12 bottles and each soaked with 25 ml. distilled water for 2 hours. 3% aqueous solution of the major constituents of babul, viz., (+)-leucocyanidin gallate, polymeric tan frac-

tions T_1 and T_2 and catechin were prepared. 50 ml. of each of these prepared solution was pipetted out into 3 bottles separately and shaken for 15 minutes after which they were left undisturbed. One bottle from each was removed after 1 hour, 3 hours and 24 hours, filtered and a known volume of the filtrate was evaporated to dryness for determining the total solubles of the liquor. The initial total solubles content of the liquor was determined by evaporating an aliquot of the liquor to dryness. The difference in weight gave the amount absorbed by 1.0 g. hide powder.

Preferential affinity of the constituents towards hide powder

One g. each of (+)-leucocyanidin gallate, (+)-catechin and polymeric tan fractions T_1 and T_2 were taken together in 200 ml. water. The liquor was consecutively treated seven times with 1.5 g. hide powder (on dry weight basis) for

Table 1

T_s GIVEN BY THE CONSTITUENTS AND GROUP FRACTIONS OF BABUL

Constituent and group fractions	T_s (°C)
Quercetin	65
Catechin	63
<i>epi</i> -catechin	64
Dicatechin	67
Gallic acid	63
Leucocyanidin gallate	79
Polymeric tan fraction (T_1)	74
Polymeric tan fraction (T_2)	75
Ether solubles of acetone extract	70
Ethyl acetate solubles of acetone extract	76
Ether and ethyl acetate insolubles of acetone extract	82
Sausage casing (untanned)	62

Table 2
AFFINITY OF PHENOLIC CONSTITUENTS OF BABUL TOWARDS HIDE POWDER

Constituents	Amount absorbed per 100 g. hide powder (in grams)			Amount absorbed in 1 hour
	1 hour	3 hours	24 hours	Amount absorbed in 24 hours
				× 100
Leucocyanidin gallate	51.5	59.5	65.2	78.7
Tan fraction (T ₁)	39.2	49.7	58.5	67.0
Tan fraction (T ₂)	35.4	44.9	57.9	61.0
Catechin	21.3	24.5	25.8	82.0

15 minutes each. The filtrate after each treatment was studied chromatographically using 6% acetic acid as solvent for first dimension and sec.-butanol: acetic acid: (14:1:5) as solvent for second dimension prior to developing with diazotised para-nitraniline. From the chromatograms so obtained, the relative affinity of these constituents was inferred.

Results and discussion

Tanning potency of individual constituents and group fractions

Quercetin, (+)-catechin, (+)-epi-catechin and gallic acid yielded practically no rise in shrinkage temperature as compared to control (Table 1). But (+)-leucocyanidin gallate showed a high T_s (79°C). Two polymeric tan fractions also showed a slightly higher T_s (74 & 75°C) but lower than the value given by (+)-leucocyanidin gallate. The ether solubles of acetone extract of babul, which contain (+)-catechin, (–)-epi-catechin, quercetin and gallic acid were found to yield a slightly higher

T_s (70°C) indicating that though individually they do not effect a rise in T_s , collectively they possess slight tanning potency. The ethyl acetate solubles of acetone extract of babul, which contain (+)-leucocyanidin gallate as a major constituent with small amounts of (+)-catechin, quercetin, gallic acid and traces of two polymeric tan fraction, were found to yield a T_s of 76°C, which is lower than that given by (+)-leucocyanidin gallate but higher than that given by the two polymeric tan fraction. In this case, obviously the effect of (+)-leucocyanidin gallate is more than those of other constituents. The ether and ethyl acetate insolubles of the acetone extract of babul containing two polymeric fractions as major constituents with traces of (+)-leucocyanidin gallate, quercetin, (+)-catechin and gallic acid yielded quite a high T_s (82°C) which is 3°C more than that given by (+)-leucocyanidin gallate, indicating as mentioned before that collectively the constituents yielded a higher T_s . Hence (+)-leucocyanidin gallate along with the two polymeric tan fractions might

be considered as mainly responsible for the tanning potency of babul infusion.

Affinity of the constituents towards hide powder

The nontans left in babul aqueous infusion after the removal of tannins by hide powder (by the official method),⁷ when concentrated carefully under vacuum and subjected to chromatographic study, indicated the presence of (+)-catechin, (-)-*epi*-catechin, (+)-dicatechin, quercetin and gallic acid, thereby confirming the removal of (+)-leucocyanidin gallate and the two polymeric tan fraction in full. It was already observed that (+)-leucocyanidin gallate and the two polymeric tan fractions T₁ and T₂ along with catechin account for 80-85% of the phenolic constituents present in babul.⁸ Hence for the affinity studies only the four major constituents of babul, viz., (+)-leucocyanidin gallate, polymeric tan fractions T₁ and T₂ and (+)-cate-

chin were taken. The results are given in Table 2.

It could be seen from the results that (+)-leucocyanidin gallate has got more affinity towards hide powder than the two polymeric tan fractions (T₁ and T₂), the affinity being appreciably more during the first hour. In fact, about 79% of the total (+)-leucocyanidin gallate absorbed in 24 hours was absorbed within 1 hour as against 67% absorbed in 1 hour in the case of T₁ and 61% in the case of T₂. Even though the absorption of (+)-catechin by hide powder is low, it is interesting to note that 82% of the total catechin absorbed in 24 hours is absorbed in 1 hour. Since (+)-leucocyanidin gallate and (+)-catechin are both mobile constituents, more than three-fourths of the total absorbables are absorbed in the first hour itself, whereas in the case of immobile constituents (T₁ and T₂) the absorption is well spread over the entire period of contact (24 hours).

Table 3
CHROMATOGRAPHIC DATA

Treatment with hide powder	Spot numbers
After first treatment	T ₁ , T ₂ , 2, 3
After second treatment	T ₁ *, T ₂ *, 2, 3
After third treatment	T ₁ *, T ₂ *, 2*, 3
After fourth treatment	T ₁ *, T ₂ *, 2*, 3
After fifth treatment	T ₁ **, T ₂ **, 2*, 3
After sixth treatment	2**, 3
After seventh treatment	3

*Intensity faint; **Intensity very faint.

Symbols denote: Polymeric tan fraction T₁: T₁; Polymeric tan fraction T₂: T₂; Leucocyanidin gallate: 2; Catechin: 3.

Preferential affinity of the constituents towards hide powder

The chromatographic data of the residual liquor after each treatment are given in Table 3. It could be observed from the results that both polymeric tan fractions T_1 and T_2 and (+)-leucocyanidin gallate are preferentially taken up by hide powder, even though the affinity of T_1 and T_2 was found to be more. It was very difficult to differentiate between the affinity of the two polymeric tan fractions T_1 and T_2 as a trailing spot was obtained and the intensity was more or less the same throughout the full length of the trailing. Anyhow, the affinity of these constituents towards hide powder when taken in equal amounts may be given as $T_1 = T_2 > (+)\text{-leucocyanidin gallate} > (+)\text{-catechin}$. Even though hide powder was found to absorb more of (+)-leucocyanidin gallate than the polymeric tan fractions T_1 and T_2 when the constituents are taken individually, the preferential affinity of the two polymeric tan fractions towards hide powder when taken together with (+)-leucocyanidin gallate should be due to the bigger particle size of the two constituents (T_1 and T_2).

Thus among the phenolic constituents present in babul, (+)-leucocyanidin

gallate and the two polymeric tan fractions T_1 and T_2 which almost form the entire tanning component were found to be responsible for the tanning potency of babul liquor with fairly good affinity towards hide powder.

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